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Pleistocene isolation caused by sea-level fluctuations shaped genetic characterization of *Pampus minor* over a large-scale geographical distribution

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Abstract

The southern lesser pomfret (*Pampus minor*) is an economically important fish, and its numbers are declining because of overfishing and environmental pollution. In addition, owing to the similarities of its external morphological characteristics to other species in the genus *Pampus*, it is often mistaken for grey pomfret (*P. cinereus*) or silver pomfret (*P. argenteus*) juveniles. In this study, the genetic diversity and structure of 264 *P. minor* individuals from 11 populations in China and Malaysia coastal waters were evaluated for the first time, to the best of our knowledge, using mitochondrial cytochrome b fragments. The results showed that *P. minor* had moderate haplotype diversity and low nucleotide diversity. Furthermore, two divergent lineages were detected within the populations, but the phylogenetic structure corresponded imperfectly with geographical location; thus, the populations may have diverged in different glacial refugia during the Pleistocene low sea levels. Analysis of molecular variation (AMOVA) showed that genetic variation originated primarily from individuals within the population. Pairwise F_{ST} results showed significant differentiation between the Chinese and Malaysian populations. Except for the Xiamen population, which was classified as a marginal population, the genetic differentiation among the other Chinese populations was not significant. During the Late Pleistocene, *P. minor* experienced a population expansion event starting from the South China Sea refugium that expanded outward, and derivative populations quickly occupied and adapted to the new habitat. The results of this study will provide genetic information for the scientific conservation and management of *P. minor* resources.

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Keywords
Cytochrome b, genetic diversity, genetic structure, South China Sea, southern lesser pomfret

Introduction

Because of the rise in fishing pressure, habitat destruction, and global climate change, understanding the level of marine biological variation and its genetic structure is of crucial significance to the protection of marine biological resources and genetic diversity (Butchart et al. 2010). Current research on population genetics mainly includes detecting the level of genetic diversity and population genetic structure within different species, estimating the effective population size, and investigating the mechanisms underlying various evolutionary factors (Ellegren 2014).

Pampus minor Liu & Li, 1998 is an offshore warm-water pelagic fish classified under the class Actinopterygii, order Perciformes and family Stromateidae. It is a newly discovered species, distributed primarily south of the mid-southern East China Sea and along the coast of Southeast Asian countries (Li et al. 2019a). Owing to the similarities in the external characteristics of *Pampus* species and the small size of *P. minor* (adults generally do not exceed 150 mm), this species was consistently mistaken for grey pomfret (*P. cinereus*) or silver pomfret (*P. argenteus*) juveniles in early studies (Liu and Li 1998).

The region in which *P. minor* is distributed experienced a series of glacial-interglacial cycles in the Late Quaternary. During glacial periods, fluctuations in sea levels led to massive changes in the area and structure of marginal seas (Wang 1999), which transformed the Western Pacific Ocean into an ideal marine region for studying how glacial periods affected marine life. We postulate that during the Last Glacial Maximum (LGM), *P. minor* was also strongly affected by the Pleistocene glacial period. Thus, under the harsh environmental conditions of the glacial period, most of the individuals within its distribution range went extinct, and only a handful of isolated populations in glacial refugia (such as the South China Sea) survived. As the climate warmed during interglacial periods, sea levels rose, which led to population expansion; hence, the corresponding phylogeographic patterns and population genetic structure may be detected.

There have been few studies on *P. minor* thus far, which have only focused on morphology (Liu and Li 1998; Li et al. 2019a), population genetics (Li et al. 2019b), and phylogenetics (Cui et al. 2010; Guo et al. 2010; Li et al. 2019a). To the best of our knowledge, no basic research has been conducted on the status and distribution of *P. minor* fishery resources, and there have been no reports analyzing the large-scale genetic structure of its distribution range. Given the general decline in fishery resources, *P. minor* resources have also been shrinking, and there is a need to understand its genetic diversity, genetic structure, effective population size, and other population genetic characteristics. These parameters form the basis for formulating strategies for

the effective protection and rational exploitation and utilization of marine fishery resources (Funk et al. 2012).

In this study, mitochondrial DNA sequences (cytochrome b, Cytb) were used to study the genetic diversity, genetic structure, and historical demography of 11 *P. minor* populations in China and Malaysia coastal waters. In addition, the effects of paleoclimatic, paleo-geological, marine geological, environmental and other factors on population formation, distribution and expansion routes, as well as genetic exchange, were revealed. This enabled us to investigate the mechanisms underlying the current phylogeographic patterns of this species, which can serve as a scientific reference for fishery management.

Materials and methods

Sample collection

Between May 2016 and December 2017, a total of 264 *P. minor* individuals from 11 geographical populations along the coasts of China (Xiamen, Zhangpu, Taiwan, Zhuhai, Zhanjiang, Beihai, Weizhou Island, Haikou, Sanya) and Malaysia (Kuala Selangor, Mukah) were collected (Fig. 1, Table 1). To ensure the accuracy of the taxonomy, the morphological identification of all specimens was based on Liu and Li (1998). A piece of back muscle tissue was frozen or preserved in 95% alcohol for molecular study.

DNA extraction, amplification, and sequencing

Genomic DNA of *P. minor* was extracted from muscle tissue using a Qiagen DNeasy kit. The genomic DNA was assessed by electrophoresis with a 1.5% agarose gel and qualified samples were stored at 4 °C for PCR amplification. The mtDNA cytochrome b (Cytb) was amplified with the primers L14734: 5'-AACCACCGTTGTTATTCAACT-3' (Inoue et al. 2001) and H15149: 5'-CTCAGAATGATATTTGTC-

Table 1. Information and molecular indices for *P. minor* based on mitochondrial DNA Cytb sequences.

Country	ID	Population	Number of individuals	Date	NH	NUH	*h*	π	*k*
China	XM	Xiamen Island	24	Apr. 2017	4	3	0.5391±0.1129	0.0007±0.0006	0.2917±0.2500
	ZP	Zhangpu	24	Apr. 2017	3	1	0.5942±0.0537	0.0016±0.0014	0.6667±0.5303
	TW	Taiwan	24	Oct. 2017	3	0	0.5072±0.0929	0.0013±0.0012	0.5507±0.4688
	ZH	Zhuhai	24	Dec. 2016	4	0	0.5326±0.1048	0.0015±0.0013	0.6015±0.4960
	ZJ	Zhanjiang	24	Dec. 2017	3	1	0.5399±0.0619	0.0014±0.0013	0.5725±0.4805
	BH	Beihai	24	Nov. 2016	5	1	0.6377±0.0606	0.0019±0.0016	0.7681±0.5824
	WZ	Weizhou Island	24	Nov. 2016	3	0	0.5543±0.0525	0.0014±0.0013	0.5906±0.4903
	HK	Haikou	24	Dec. 2016	5	1	0.4855±0.1129	0.0013±0.0012	0.5399±0.4629
	SY	Sanya	24	Dec. 2016	3	0	0.4891±0.0843	0.0012±0.0011	0.5145±0.4491
Malaysia	KS	Kuala Selangor	24	May 2016	7	3	0.6341±0.0973	0..49±0.0031	2.0109±1.1731
	SK	Mukah	24	May 2016	7	4	0.6087±0.1115	0.0038±0.0026	1.5652±0.9669
Total			264	–	22	14	0.6763±0.0189	0.0035±0.0023	1.4385±0.8794

Note: NH, number of haplotypes; NUH, number of unique haplotypes; *h*, haplotype diversity; π, nucleotide diversity; *k*, mean number of pairwise differences

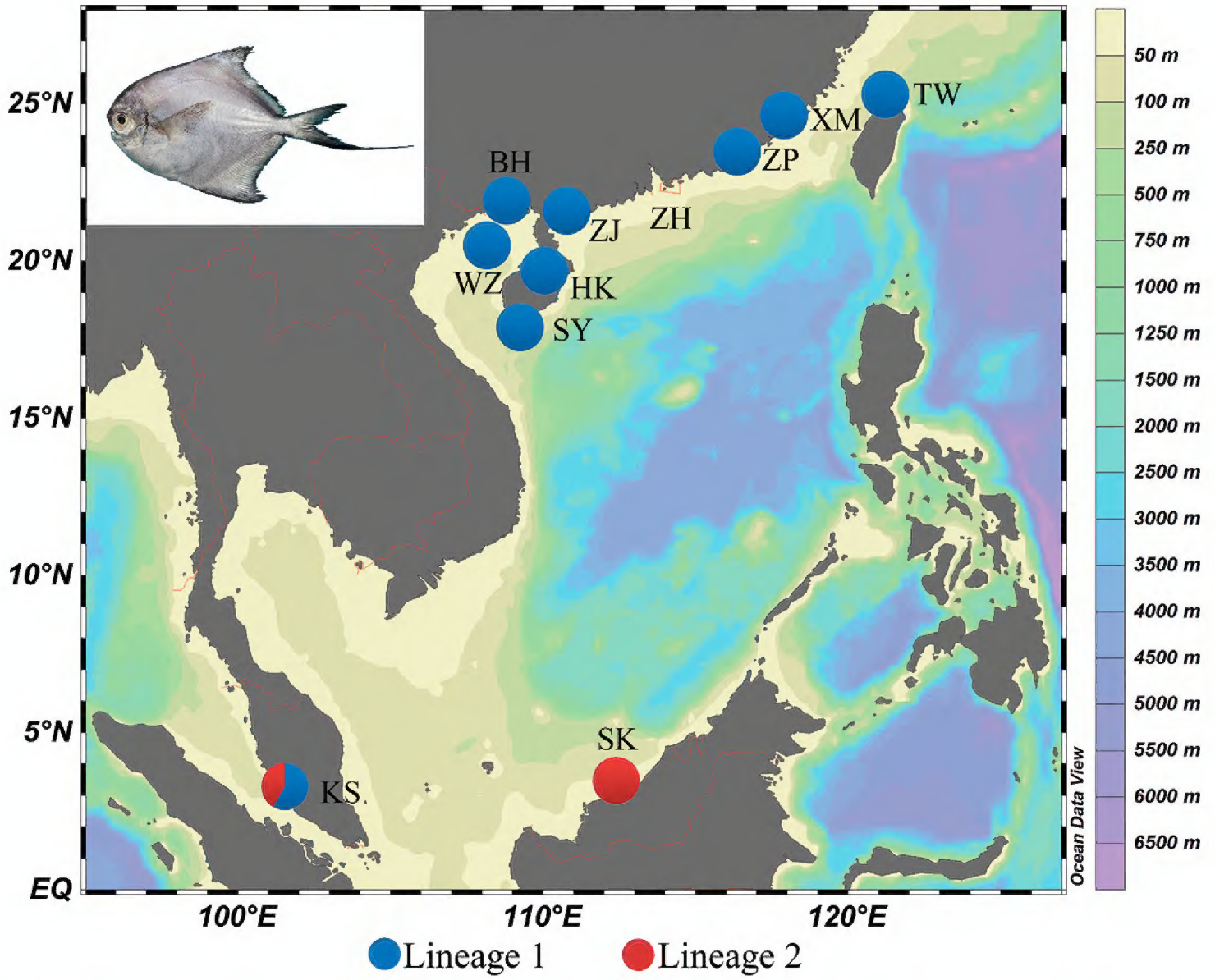


Figure 1. Sampling locations of *P. minor*. Populations are marked by abbreviations that correspond to Table 1.

CTCA-3' (Ohdachi et al. 1997). All PCR reactions were carried out in a final mixture of 25 μL: 0.15 μL Taq polymerase, 2.5 μL 10× PCR buffer, 17.5 μL ultrapure water, 2 μL dNTPs, 1 μL of forward primer (5 μM), 1 μL of reverse primer (5 μM), and 1 μL of template DNA. PCR was carried out by initial denaturation step at 94 °C for 4 min, then followed by 32 cycles of denaturation at 94 °C for 30 sec, annealing at 50 °C for 30 sec, and extension at 72 °C for 30 sec, and plus a final extension at 72 °C for 10 min. After purification of the PCR products, both DNA strands were sequenced. The newly determined Cytb sequences were deposited in GenBank under the accession numbers (MT303974–MT303978, MF616364–MF616380).

Data analysis

The Cytb sequences were aligned using the DNASTAR (Madison, WI, USA) software and manually edited. Haplotypes were defined based on sequence data without considering sites with gaps using DnaSP ver. 5.00 (Librado and Rozas 2009). Genetic diversity in each population was accessed as polymorphic sites, haplotype number, mean number of pairwise differences, haplotype diversity, and nucleotide diversity us-

ing ARLEQUIN version 3.5 (Excoffier et al. 2005). Analysis of molecular variation (AMOVA) in ARLEQUIN software was employed to investigate the genetic variation and test population structure. The MEGA 5.0 (Tamura et al. 2011) was applied to reconstructed the neighbor-joining (NJ) tree based on the genetic distance among haplotypes, and implemented with 1000 replicates. The relationships of haplotypes by unrooted minimum spanning tree (MST) was evaluated via the MINSPNET algorithm in ARLEQUIN software (Excoffier et al. 2005), and the MST topological structure was subsequently drawn by hand.

Both neutrality testing and mismatch distribution analysis were used to infer the historical demography expansions, as implemented in ARLEQUIN. Deviations from neutrality, significant negative values of Fu's *Fs* and Tajima's *D* statistic, were evaluated to experience population growth and spatial range expansion. A molecular clock-based time estimate provided an approximate timeframe for evaluating phylogeographical hypotheses. Historical demographic expansions were further tested by nucleotide mismatch distribution, based on three parameters: θ_0, θ_1 (θ before and after population growth), and τ (time since expansion, expressed in units of mutational time) (Rogers and Harpending 1992). The real-time since expansion was computed by the equation $\tau=2\times\mu\times t$, where μ is the mutation rate for the whole sequence and t is the time since expansion.

In the present study, a sequence divergence rate of 0.2×10^{-7}/site/year (Avise 1998; Sun and Tang 2018) was applied to the Cytb sequences of *P. minor*. Bayesian skyline plots were created using BEAST v.8 (Drummond et al. 2012). However, a molecular clock-based time estimate provided an approximate time frame for evaluating phylogeographic hypotheses.

Results

Genetic diversity

A total of 264 sequences were obtained from the 11 *P. minor* populations. After manual alignment, a target fragment of 415 bp was obtained, of which there were 19 polymorphic sites, 12 singleton sites, seven parsimony informative sites, and no indels. A+T content (62.32%) was significantly higher than G+C content, thus showing an AT bias.

A total of 22 Cytb haplotypes were defined in the 264 individuals. The number of haplotypes in each population ranged from three to seven. The number of haplotypes shared by two or more populations was eight (36.4%). There were 14 unique haplotypes (63.6%), and seven populations had unique haplotypes, with the number ranging from one (ZP, ZJ, BH, HK) to four (SK) (Table 1). There were large differences in the composition and distribution of haplotypes between the Chinese and Malaysian populations, with only one haplotype shared between the two. Both the Chinese (7/12=58.3%) and Malaysian (7/11=63.6%) populations were dominated by unique haplotypes (Fig. 2). The haplotype with the highest frequency was Hap_2, consisting

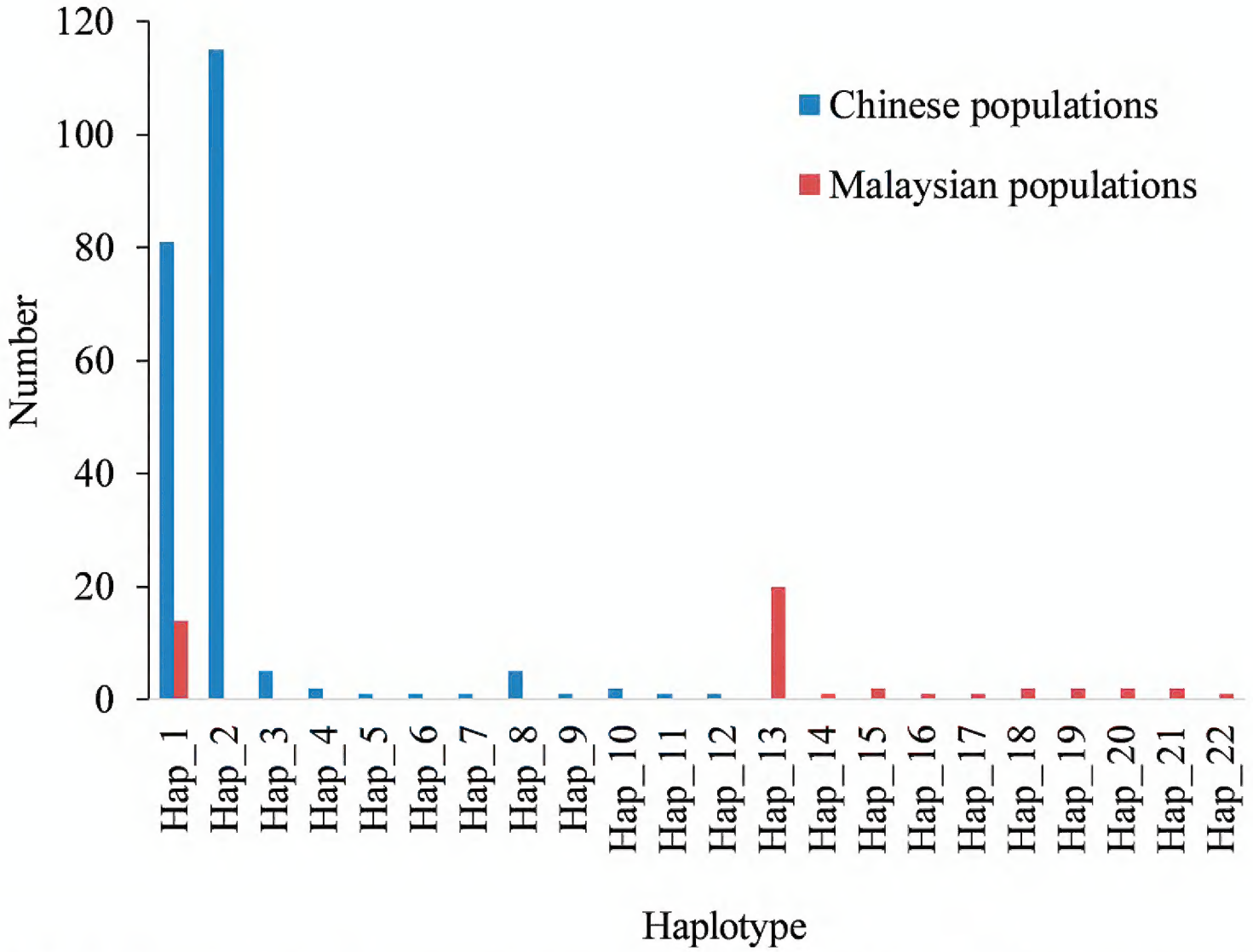


Figure 2. Composition and distribution of 22 Cytb haplotypes in the Chinese and Malaysian populations.

of 115 sequences. Hap_1, consisting of 95 sequences, was the only haplotype shared by the Chinese and Malaysian populations and may be an ancestral haplotype.

In general, the *P. minor* populations exhibited moderate haplotype diversity (0.6763 ± 0.0189) and low nucleotide diversity (0.0035 ± 0.0023). This phenomenon is usually due to bottleneck effects, resulting in population expansion or rapid population growth in small populations, accompanied by the generation of a large number of new mutations (Avise et al. 1984; Grant and Bowen 1998).

Genetic structure

An NJ tree was constructed based on the 22 *P. minor* Cytb haplotypes. The results showed two divergent lineages detected within the populations but with low bootstrap values. The phylogenetic structure detected corresponded imperfectly to the geographical locations (Fig. 3). Lineage 1 was composed of 12 haplotypes (230 individuals), and lineage 2 was composed of ten haplotypes (34 individuals) (Fig. 3). Lineage 1 was composed of all the Chinese populations and some individuals from Malaysia KS, whereas lineage 2 was composed entirely of Malaysian populations.

An unrooted MST was constructed based on the 22 Cytb haplotypes of the Chinese and Malaysian populations (Fig. 4). All sequences exhibited multiple primary haplotypes, and the other haplotypes were radially distributed around the primary

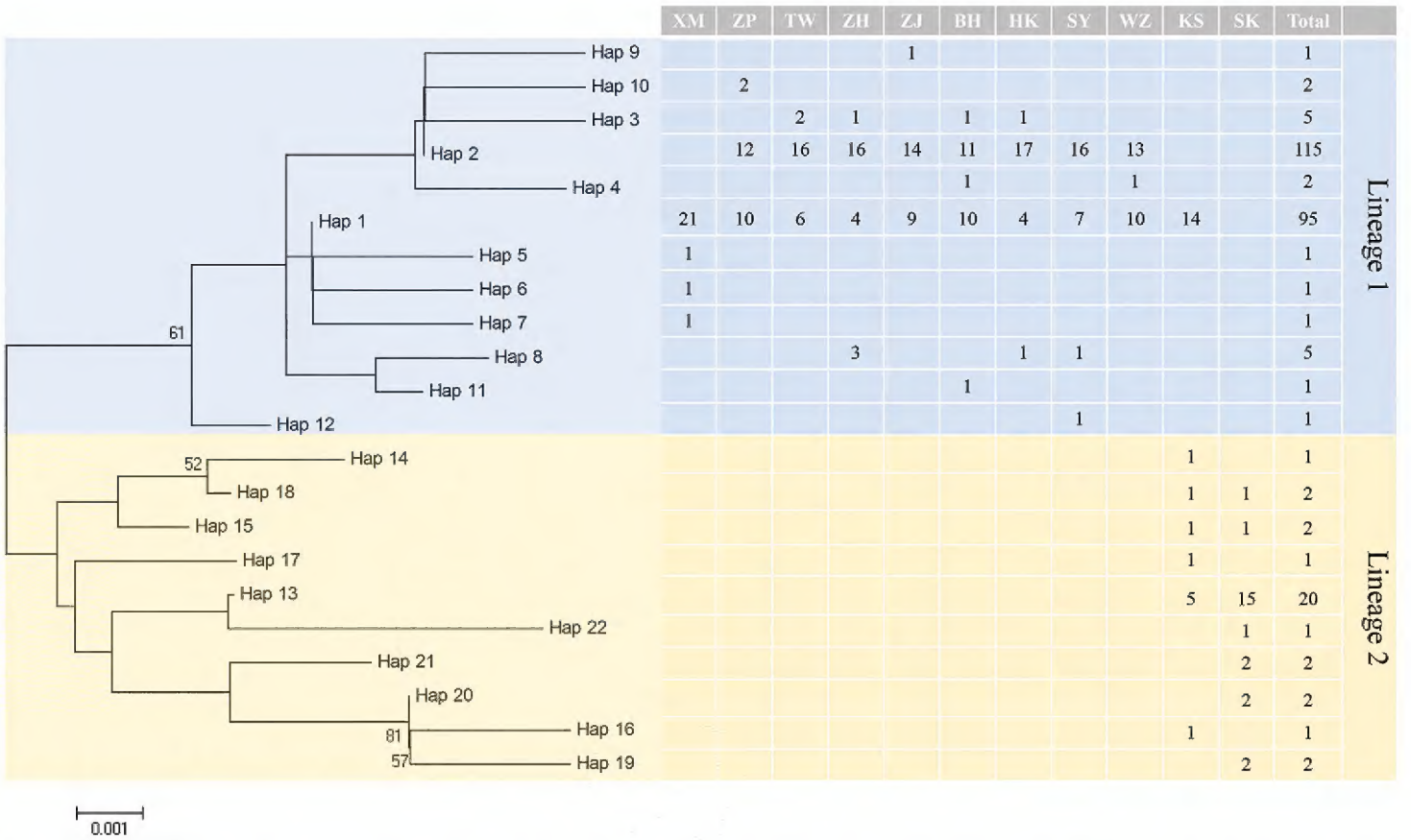


Figure 3. NJ tree and distribution of Cytb haplotypes among populations for *P. minor*. Bootstrap supports of > 50 in 1000 replicates are shown.

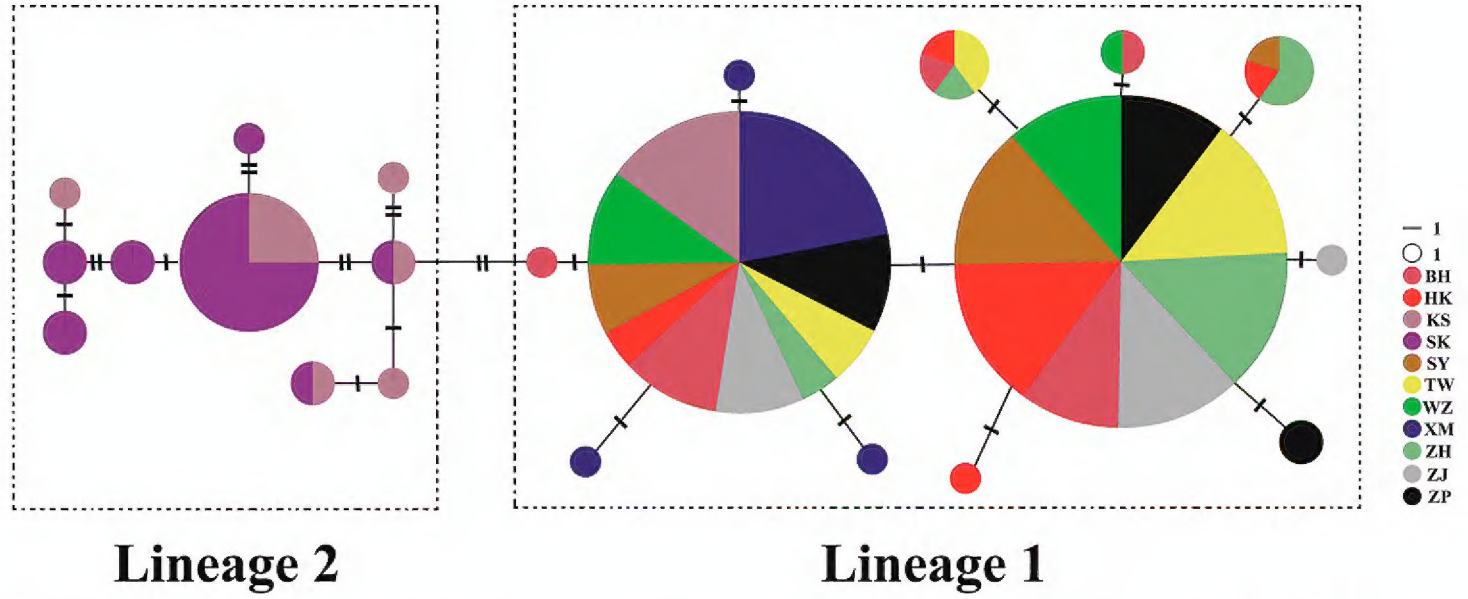


Figure 4. Unrooted minimum spanning tree showing the genetic relationships among the Cytb haplotypes of *P. minor*. Circle sizes are proportional to haplotype frequency. Perpendicular tick marks on the lines joining the haplotypes represent the number of nucleotide substitutions.

haplotypes with obvious phylogenetic structures that corresponded to the Chinese and Malaysian populations.

Based on the TrN+G model, the net genetic distance between the two haplotype lineages was 0.006. Based on a mitochondrial Cytb sequence divergence rate of 2% per million years, the time of divergence between lineages 1 and 2 was approximately 300 thousand years ago (Kya).

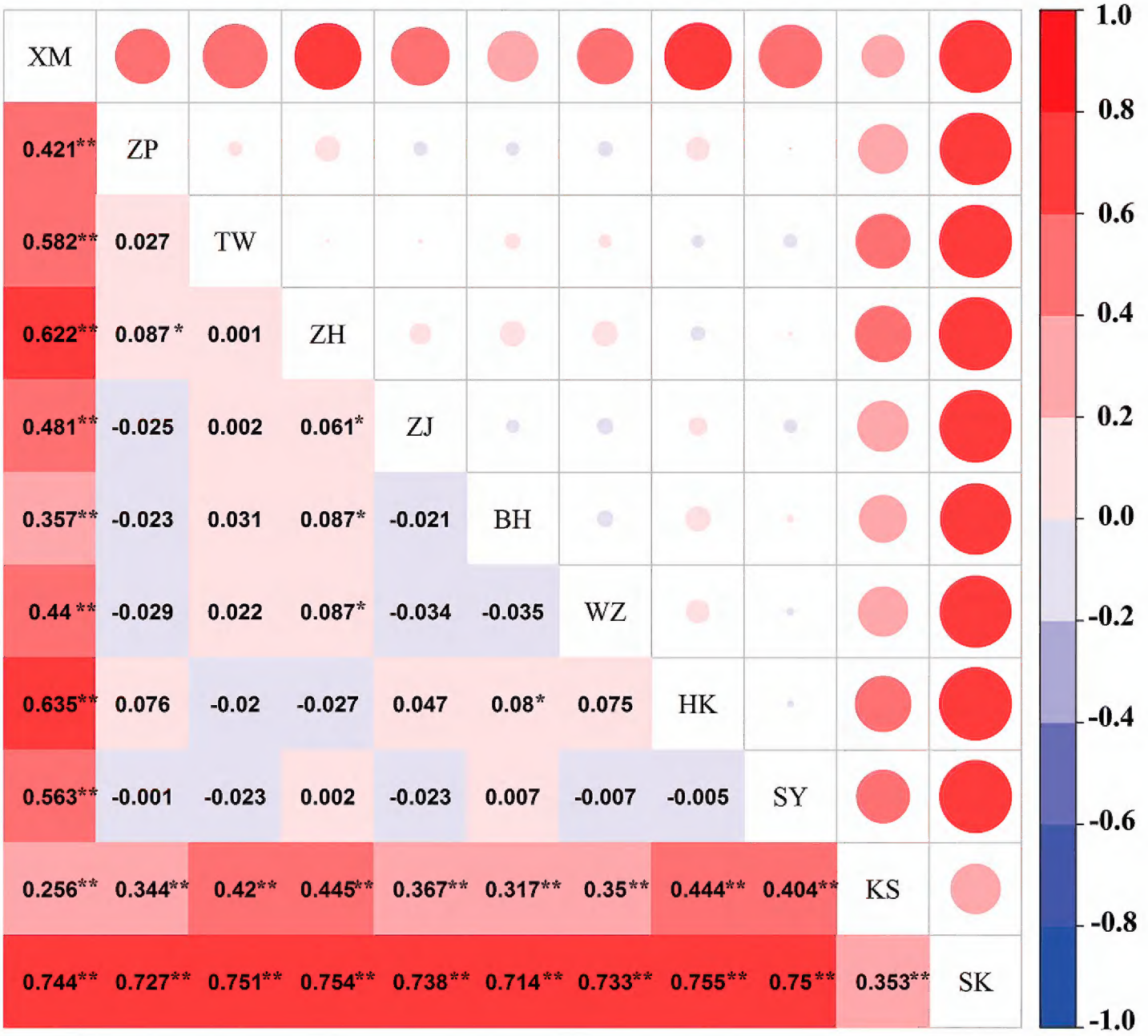


Figure 5. Matrix of pairwise F_{ST} values between 11 *P. minor* populations based on Cytb sequences. * significant at $p < 0.05$ by the permutation test, * * extremely significant at $p < 0.01$ by the permutation test.

The fluctuation ranges of pairwise F_{ST} between populations were relatively large. The F_{ST} values between the Chinese and Malaysian populations were all above 0.25, and statistical tests indicated significance, thus showing very great differentiation (Wright 1965) (Fig. 5). The F_{ST} values between the Xiamen and other populations were relatively large, and differentiation was statistically significant. Except for the Xiamen population, the F_{ST} values between the Chinese populations were below 0.15, thus showing limited differentiation, and most of the statistical tests were not significant. Some F_{ST} values among the *P. minor* populations were still negative, suggesting that the level of differentiation within *P. minor* populations was greater than that among populations (Aris-Brosou and Excoffier 1996).

AMOVA was used to detect the genetic structure of the populations (Table 2). First, all *P. minor* populations were analyzed as one gene pool. The results showed significant genetic differentiation within each population ($\Phi_{ST} = 0.477$, $p < 0.01$), ac-

Table 2. AMOVA analysis of *P. minor* populations based on mitochondrial Cytb sequences.

Source of variation	Sum of squares	Percentage	Φ statistic	*p*
One gene pool (XM, ZP, TW, ZH, ZJ, BH, WZ, HK, SY, KS and SK)				
Among populations	89.92	47.74	Φ_{ST}=0.477	0.00
Within populations	99.25	52.26		
Two gene pools (XM, ZP, TW, ZH, ZJ, BH, WZ, HK and SY) (KS and SK)				
Among groups	64.23	30.74	Φ_{CT}=0.612	0.01
Among populations within groups	25.69	8.04	Φ_{SC}=0.207	0.00
Within populations	99.25	61.23	Φ_{ST}=0.693	0.00
Four gene pools (TW) (XM, ZP, ZH, ZJ, BH and WZ) (HK and SY) (KS and SK)				
Among groups	67.08	40.30	Φ_{CT}=0.403	0.03
Among populations within groups	22.83	13.95	Φ_{SC}=0.234	0.00
Within populations	99.25	45.75	Φ_{ST}=0.542	0.00
Six gene pools (TW) (XM, ZP, ZH and ZJ) (BH and WZ) (HK and SY) (KS) (SK)				
Among groups	79.87	42.54	Φ_{CT}=0.425	0.04
Among populations within groups	10.05	8.43	Φ_{SC}=0.147	0.00
Within populations	99.25	49.03	Φ_{ST}=0.510	0.00

counting for 52.26% of the variation, whereas genetic differentiation among populations accounted for only 47.74% of the variation.

To further confirm the genetic structure of the *P. minor* populations, the 11 populations were grouped into two, four, and six gene pools based on their geographic distribution. The results of all groupings showed that the genetic differentiation among groups was relatively large with statistical significance ($p < 0.05$), whereas genetic differentiation originating primarily within populations was highly significant ($p < 0.01$), and genetic differentiation among populations within groups was also significant ($p < 0.01$).

Historical demography

Two haplotype lineages were detected in all Chinese and Malaysian populations with imperfect geographic lineage structures. Due to the significant differentiation among all populations, the historical demography of the two haplotype lineages was analyzed. The nucleotide mismatch distribution in all *P. minor* sequences was unimodal, and similar results were found in both lineages (Fig. 6). Neutrality test results showed that the Fu's F_S tests for each lineage and the overall population yielded negative values, and were statistically significant ($p < 0.05$). Tajima's *D* test for each lineage and the

Table 3. Summary of molecular diversity, neutral test and goodness-of-fit test for *P. minor*.

	Number	NH	*h* ± SD	*π* ± SD	*k* ± SD	Tajima's *D*		Fu's *Fs*		Goodness-of-fit test				
						D	*p*	*Fs*	*p*	τ	θ_0	θ_1	*SSD*	*HRI*
Lineage 1	230	7	0.5807 ± 0.0177	0.0016 ± 0.0013	0.6643 ± 0.5131	-1.378	0.044	-7.647	0.003	0.836	0.011	83022	0.0258ns	0.1889ns
Lineage 2	34	7	0.6524 ± 0.0917	0.0042 ± 0.0028	1.7273 ± 1.0316	-0.905	0.189	-3.499	0.030	1.813	0.000	2.601	0.0321ns	0.1151ns
All	264	22	0.6763 ± 0.0189	0.0035 ± 0.0023	1.4385 ± 0.8794	-1.379	0.045	-12.923	0.001	1.000	0.000	99999	0.026ns	0.109ns

Note: NH, numbers of haplotypes; *h*, haplotype diversity; *π*, nucleotide diversity; *k*, average number of pairwise differences; ns, $p > 0.05$

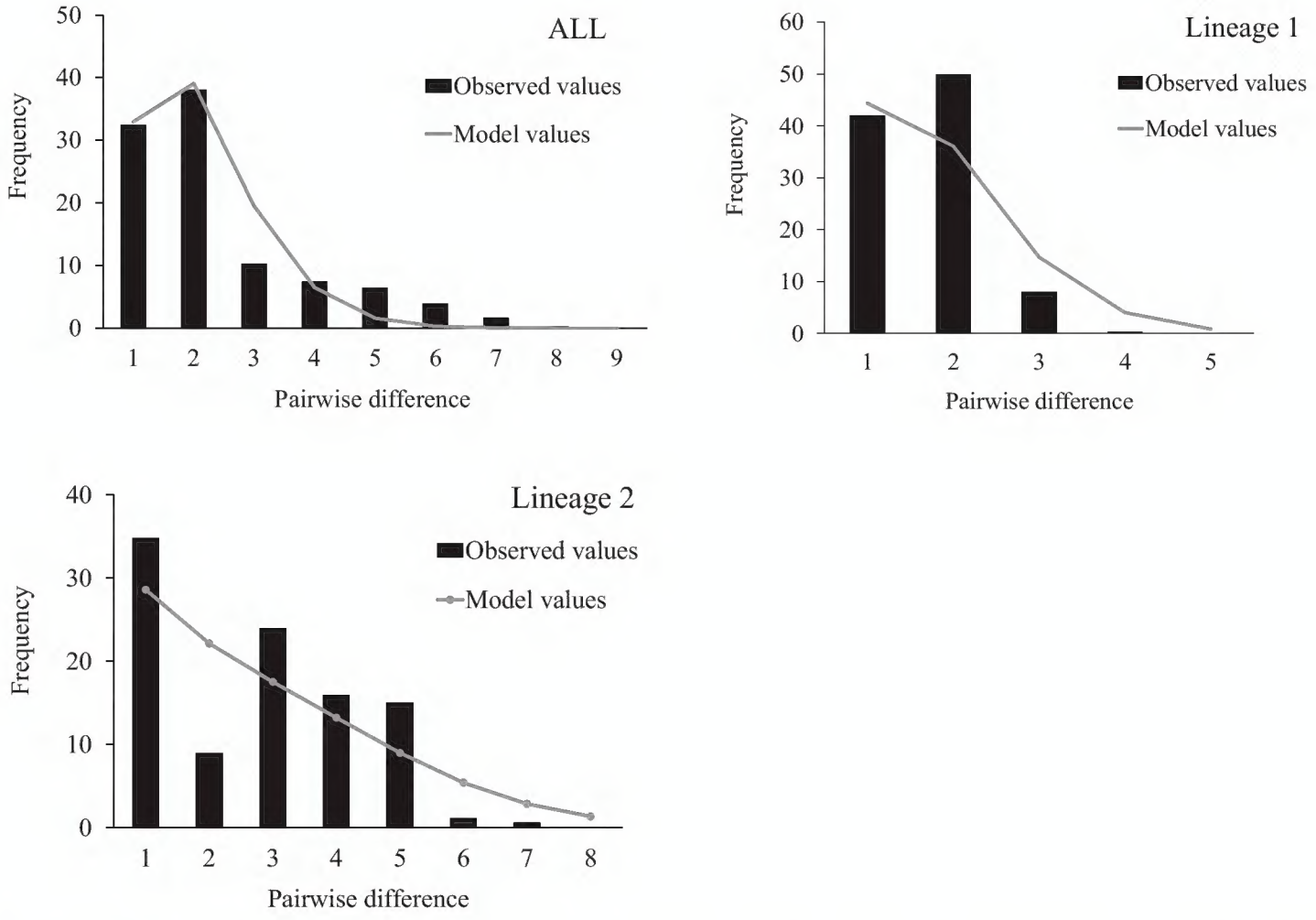
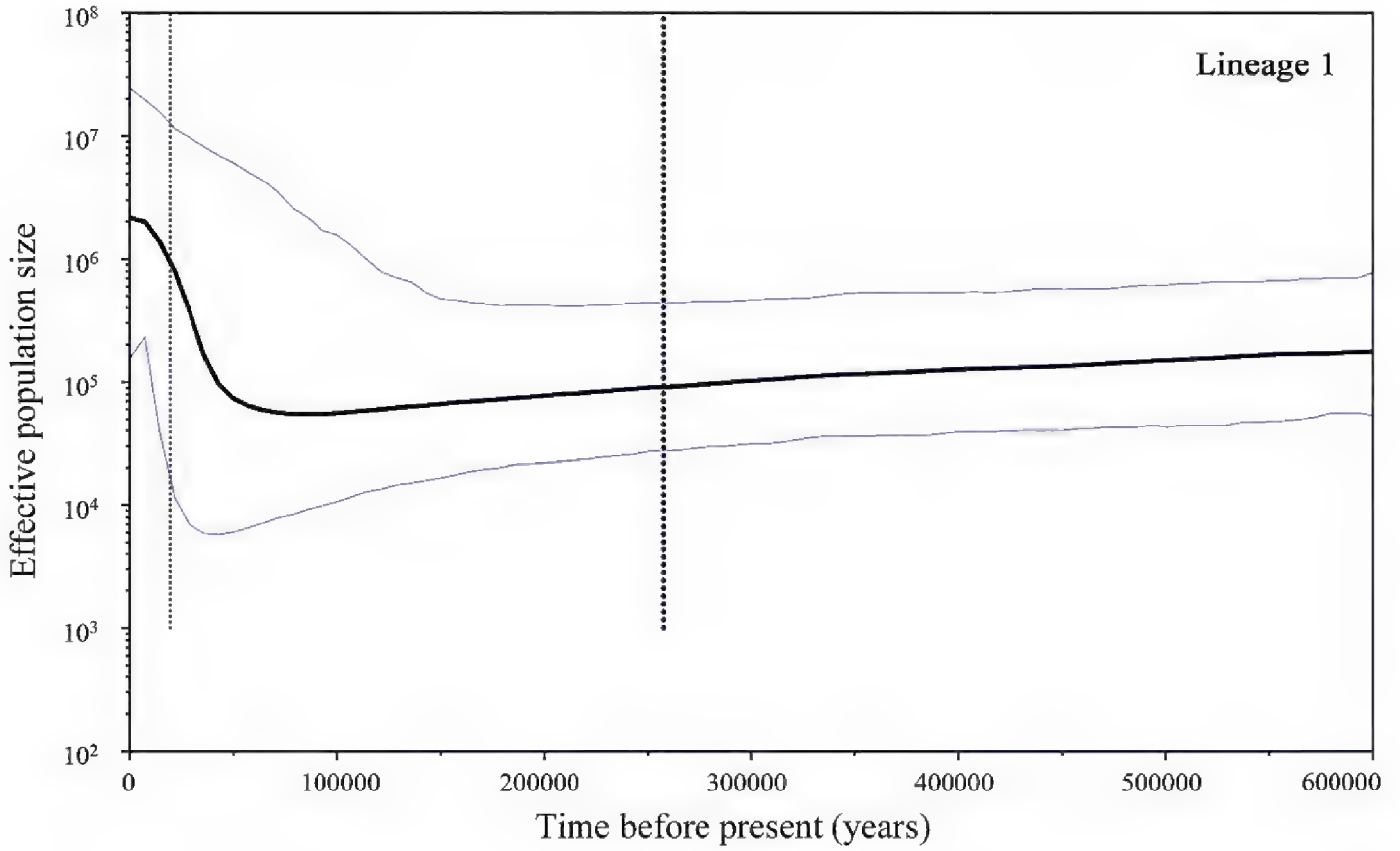


Figure 6. The expected mismatch distributions under a sudden expansion model (solid gray line) and the observed pairwise difference (black bars) of Cytb haplotypes of *P. minor*.

overall population yielded negative values, and were all statistically significant, except for lineage 2. In addition, the *SSD* and *HRI* test indices were not significant ($p > 0.05$) (Table 3), indicating that there was no significant deviation from the expected distribution under the population expansion model. Therefore, this can be used to analyze the historical demography of the *P. minor* populations, implying that this species has experienced population expansion events. Simultaneously, the Bayesian skyline plot (BSP) also indicated the same result (Fig. 7).

The τ value of the nucleotide mismatch distribution provides a time point for estimating population expansion. The τ value of lineage 2 was 1.813 (95% CI: 0.059–5.600), which was larger than that of lineage 1 (0.836, 95% CI: 0.572–1.357) (Table 3). Based on a divergence rate of 0.2×10^{-7}/site/year and τ, the population expansion time points of lineages 1 and 2 were estimated to be 101 and 218 Kya, respectively, which was during the Late Pleistocene. The ratio of θ_1 after expansion to θ_0 before expansion is infinite, indicating a sharp increase in the size of the effective maternal population of *P. minor* after population expansion. BSPs for lineage 1 and lineage 2 showed that both lineages have undergone the Late Pleistocene demographic expansion (Fig. 7), which started at different times. The effective population size of lineage 2 increased slowly, while the effective population size of lineage 1 increased sharply after the LGM.



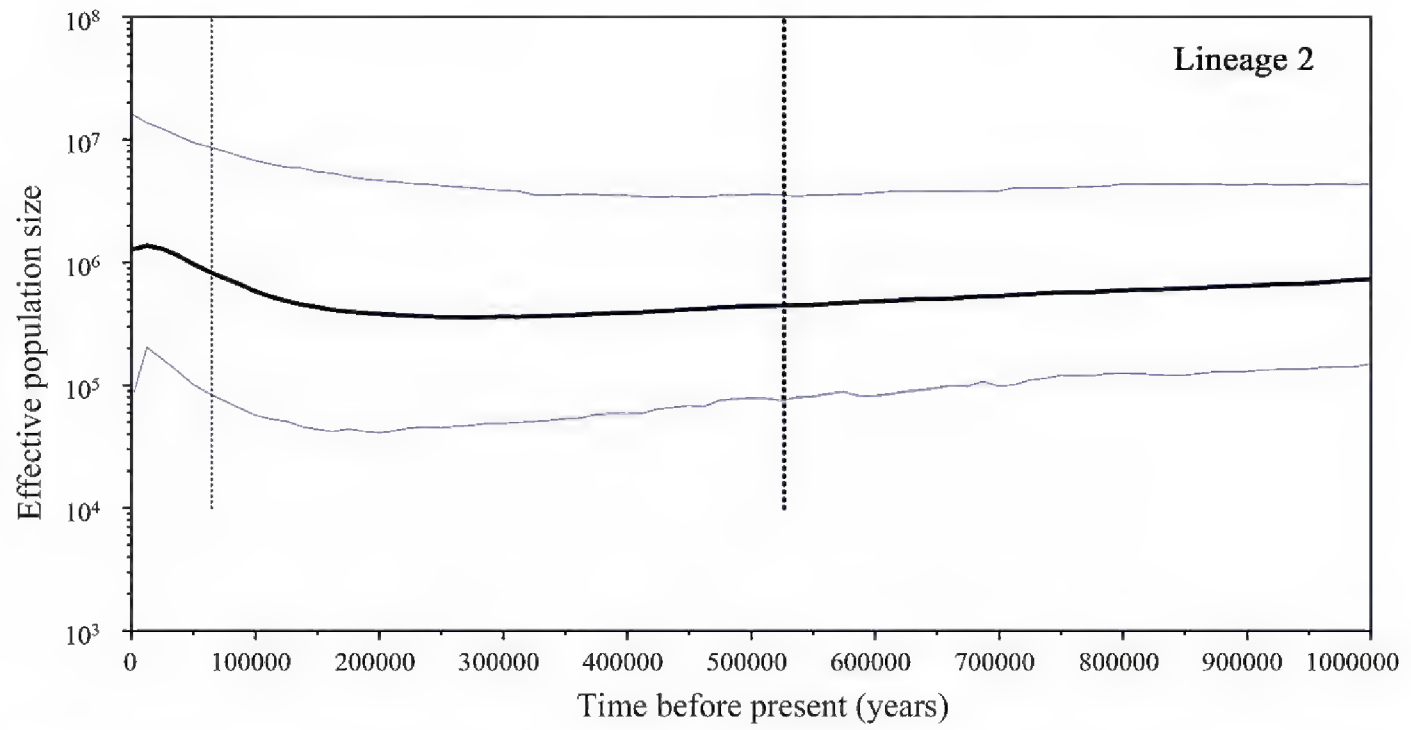


Figure 7. BSPs showing N_{ef}T (N_{ef}= effective population size; T = generation time) changes over time for *P. minor* based on Cytb sequences. The upper and lower limits of the blue line represent the 95% confidence intervals of highest posterior densities (HPD) analysis. The solid black line represents median estimates of N_{ef}T.

Discussion

Genetic diversity is the basis of both species and ecological diversity, while species and genetic diversity are both the basis of ecosystem diversity. Studies on the genetic diversity of species have attracted increasing attention from domestic and international researchers. The genetic diversity of a species directly affects its adaptation to the environment: the higher its level of diversity, the greater its potential for evolution and

the stronger its adaptation to environmental changes, whereas the opposite implies the possibility of its deterioration or extinction (Rosel et al. 1995).

Compared to the levels of intraspecific genetic diversity of Cytb gene sequences in *Trachidermus fasciatus* (h = 0.97 ± 0.011) (Gao et al. 2013), *P. argenteus* (h = 0.775 ± 0.041) (Zhao et al. 2011a), *Anguilla mossambica* (0.691 ± 0.043) (Frankowski et al. 2020) and other fishes, the genetic diversity of Chinese and Malaysian *P. minor* was at a moderate level. From a historical evolutionary perspective, large population sizes, environmental heterogeneity and life-history traits that favor rapid population increases are the main reasons for maintaining high haplotype diversity in natural populations of marine fishes. *Pampus minor* is widely distributed in the mid-southern East China Sea, South China Sea, and the coastal regions of Southeast Asian countries, indicating that large population sizes may account for the relatively high levels of haplotype diversity observed in this study. However, little is known about these life-history traits for *P. minor*, and further study is needed to examine this correlation. In any case, the *P. minor* populations had moderate haplotype diversity and low nucleotide diversity. Fish populations with this type of diversity pattern may have experienced historical expansion events, and a population bottleneck followed by rapid population growth and accumulation of mutations (Grant and Bowen 1998).

Stepien (1999) suggests that the stable number of shelf fish over the long term and a large effective population are the causes of its high haplotype diversity. Although pomfret fishery resources have declined due to overfishing (Jin et al. 2005), the larger amount of fishery resources and the number of effective populations of *P. minor* compared to other economic fishes have helped maintain its moderate level of genetic diversity. In addition, *P. minor* is a widely distributed species and has a wide dietary preference. Its spawning grounds exhibit different characteristics according to the different marine regions in which it is distributed, and its habitat conditions are heterogeneous (Wu et al. 2012). These life-history traits and the environmental heterogeneity of *P. minor* can promote rapid population growth, implying that the natural selection pressure faced by the population is relatively small, which may have led to the increased accumulation of genetic mutations and a rich genetic diversity.

The genetic diversity distribution of a species is not only affected by past historical events, but also by current evolutionary forces (e.g., migration). The discontinuity of habitats and the instability of population changes can result in differentiation between species populations (Stepien 1999). Both the NJ tree and unrooted MST showed two divergent lineages of *P. minor*. AMOVA also showed that the genetic differentiation primarily originated within populations. The time of divergence of the two lineages was approximately 300 Kya, and the different glacial refugia during Pleistocene low sea levels may have caused the divergence of the two lineages. Many previous studies have shown that Pleistocene glaciations are important factors in the genetic differentiation of many marine organisms (Liu et al. 2007; Shen et al. 2011; Zhao et al. 2011a, b; Wu et al. 2012; Gao et al. 2013).

In the Late Quaternary, the global climate experienced a series of glacial-interglacial cycles. In the last 800 Kya, climate fluctuations mainly occurred at intervals of

~100 Kya (Lambeck et al. 2002). The fourth glacial period ended at about 420 Kya (Petit et al. 1999), which coincided with the time of divergence for the two lineages of *P. minor*. With the arrival of the fourth glacial period, the sea level fell by about 120~140 m, and *P. minor* populations may have become isolated in the South China Sea refugium. After the glacial period, as sea levels rose, *P. minor* populations in the refugium expanded toward the coasts of China and Malaysia. The NJ tree and haplotype network tree show that Hap_1 was the only shared haplotype between the Chinese and Malaysian populations, and accounted for a relatively large number of individuals. This indicated that it was the ancestral haplotype, thus further demonstrating that the *P. minor* populations originated from the same refugium.

The protein encoded by the mitochondrial Cytb gene acts as a subunit in complex V of the oxidative phosphorylation pathway (Saraste 1999). Studies have shown that non-synonymous mutations in the mitochondrial Cytb gene can affect the metabolism- and energy-related selective evolution of animals (Mishmar et al. 2003; da Fonseca et al. 2008; Xu et al. 2017). In marine fishes, different dimensions of natural selection between populations may be related to temperature adaptation and aerobic exercise associated with individual size (Bélanger-Deschênes et al. 2013; Jacobsen et al. 2016; Zhang et al. 2016). These results have also been validated in studies of *Sebastiscus marmoratus* (Xu et al. 2017). Currently, *P. minor* has a wide thermal amplitude. After undergoing population expansion, Chinese and Malaysian *P. minor* populations were able to adapt to the habitats of different marine regions and withstand different natural selection pressures. With the passing of time, many new mutations appeared, and abundant haplotype diversity accumulated to form a unique haplotype. Lineage 2 was composed entirely of the haplotype of the Malaysian population, and in lineage 1, only KS appeared in the ancestral haplotype Hap_1, while all other haplotypes were from the Chinese populations. In other words, except for the ancestral haplotype, the remaining haplotypes of the Chinese and Malaysian *P. minor* populations have produced substantial differentiation, each accounting for their unique haplotypes. However, from the current perspective, there was still insufficient time for ample nucleotide variation to be produced. F_{ST} results also showed that significant genetic differentiation had occurred between the Chinese and Malaysian *P. minor* populations, further validating their respective accumulation of genetic variation to adapt to their living environments. Due to Malaysia's low latitude, high water temperature and greater number of habitats, as well as the effect of the monsoon systems, which cause the ocean currents to bring an abundance of plankton as a richer source of food, there is reduced pressure on the Malaysian *P. minor* population, allowing for the accumulation of more genetic variation, which in turn results in higher genetic diversity.

An interesting result was found in the Xiamen population, which showed significant differentiation from other populations, indicating that the breeding patterns of *P. minor* are complex. A second confirmation was performed on the sample sources and the results of the data analysis to eliminate the possible effects of these factors. Studies have reported that when *P. minor* was first discovered, the northern boundary of its

distribution range was in the Xiamen marine region, and its geographical distribution range was in the waters south of the Taiwan Strait (Liu and Li 1998). Xiamen is in the northern boundary of the *P. minor* population distribution range; that is, it is a marginal population. In studies of adaptive evolution, the marginal populations of species are more sensitive to environmental changes, exhibit more pronounced population differentiation, and genetic polymorphic sites associated with adaptive evolution are more readily detected (Bridle and Vines 2007). These phenomena have been confirmed in the Xiamen population, and this result, to some extent, also strongly supports the analysis of population genetic patterns and population evolution for marginal effects at a specific spatiotemporal scale of a single species. Xiamen Island is a semi-enclosed island surrounded by the mainland. Freshwater flows from the southeastern part of the Jiulong River and the outside are blocked by Jinmen Island, resulting in complex and variable hydrological environmental factors (Jing et al. 2011). This may also be the cause of *P. minor* differentiation in Xiamen.

Based on these results, we speculate that the time during which *P. minor* expanded from the refugium to occupy the coastal areas of China and Malaysia was relatively short. With the passage of time, the Chinese and Malaysian *P. minor* populations accumulated sufficient genetic variation to diverge completely. Similar results have been detected in the genetic structure of Chinese pomfret with similar distributions. The results of this study on the population genetics of *P. minor* are consistent with the proposed mesoscale boundary units suggested for the management of the region by Ablan et al. (2002). The Chinese coastal population should be classified as a north-central group (encompassing northwestern Taiwan, northern Vietnam, and the northwestern Philippines), and the Malaysian population should be classified as a southwestern group (comprising southern Vietnam and the eastern coast of mainland Malaysia). Unfortunately, the genetic diversity of *P. minor* in coastal China is lower than that of the Malaysian population, which is directly related to the high fishing pressure in China's offshore waters. Therefore, in order to safeguard fishery resources, emphasis should be placed on the protection of *P. minor* resources, fishing in coastal *P. minor* spawning grounds should be prohibited, and bottom trawling, gillnet and set net operations should be strictly prohibited. If these measures are taken seriously and implemented, *P. minor* resources will gradually recover, and the tragedy of resource decline of traditional commercial fishes in China, such as the large yellow croaker (*Larimichthys crocea*), small yellow croaker (*Larimichthys polyactis*), and hairtail (*Trichiurus haumela*), will been avoided.

Conclusion

Genetic signature of *P. minor* in China and Malaysia coastal waters were evaluated. The results showed that all *P. minor* had moderate haplotype diversity and two divergent lineages. The phylogenetic structure of *P. minor* corresponded imperfectly with geographical location at the Cytb gene level, but significant divergence between Chinese and Malaysian populations was detected. To get precise phylogeographic structure, more

sensitive DNA markers such as SLAF, RAD and WGS will be employed and reveal the adaptive evolution mechanism of this species. Lower haplotype diversity is detected in China, which further indicated that Chinese fishery resources are facing greater fishing pressure and more focus is needed on fishery protection and management.

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References

Ablan MCA, McManus JW, Chen CA, Shao KT, Bell J, Cabanban AS, Tuan VS, Arthana IW (2002) Meso-scale transboundary units for the management of coral reefs in the South China Sea Area. NAGA, World Fish Center Quarterly 25: 4–9. http://www.worldfishcenter.org/Naga/Naga25-3&4/pdf/NAGA_25no3n4_features_a.pdf

Aris-Brosou S, Excoffier L (1996) The impact of population expansion and mutation rate heterogeneity on DNA sequence polymorphism. Molecular Biology and Evolution 13: 494–504. https://doi.org/10.1093/oxfordjournals.molbev.a025610

Avise JC (1998) The history and purview of phylogeography: a personal reflection. Molecular Ecology 7: 371–379. https://doi.org/10.1046/j.1365-294x.1998.00391.x

Avise JC, Neigel JE, Arnold J (1984) Demographic influences on mitochondrial DNA lineage survivorship in animal populations. Journal of Molecular Evolution. 20: 99–105. https://doi.org/10.1007/BF02257369

Bélanger-Deschênes S, Couture P, Campbell PGC, Bernatchez L (2013) Evolutionary change driven by metal exposure as revealed by coding SNP genome scan in wild yellow perch (*Perca flavescens*). Ecotoxicology 22(5): 938–957. https://doi.org/10.1007/s10646-013-1083-8

Bridle JR, Vines TH (2007) Limits to evolution at range margins: when and why does adaptation fail? Trends in Ecology and Evolution 22: 140–147. https://doi.org/10.1016/j.tree.2006.11.002

Butchart SHM, Walpole M, Collen B, Van Strien A, Scharlemann JPW, Almond REA, Baillie JEM, Bomhard B, Brown C, Bruno J, Carpenter KE, Carr GM, Chanson J, Chenery AM, Csirke J, Davidson NC, Dentener F, Foster M, Galli A, Galloway JN, Genovesi P, Gregory RD, Hockings M, Kapos V, Lamarque J-F, Leverington F, Loh J, McGeoch MA,

McRae L, Minasyan A, Morcillo MH, Oldfield TEE, Pauly D, Quader S, Revenga C, Sauer JR, Skolnik B, Spear D, Stanwell-Smith D, Stuart SN, Symes A, Tierney M, Tyrrell TD, Vié J-C, Watson R (2010) Global biodiversity: indicators of recent declines. Science 328: 1164–1168. https://doi.org/10.1126/science.1187512

Cui ZX, Liu Y, Liu J, Luan WS (2010) Molecular identification of *Pampus* fishes (*Perciformes*, *Stromateidae*). Ichthyological Research 57: 32–39. https://doi.org/10.1007/s10228-009-0119-9

Da Fonseca RR, Johnson WE, O'Brien SJ, Ramos MJ, Antunes A (2008) The adaptive evolution of the mammalian mitochondrial genome. BMC Genomics 9: 1–119. https://doi.org/10.1186/1471-2164-9-119

Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution 29(8): 1969–1973. https://doi.org/10.1093/molbev/mss075

Ellegren H (2014) Genome sequencing and population genomics in non-model organisms. Trends in Ecology and Evolution 29: 51–63. https://doi.org/10.1016/j.tree.2013.09.008

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evolutionary Bioinformatics 1: 47–50. https://doi.org/10.1177/117693430500100003

Funk WC, McKay JK, Hohenlohe PA, Allendorf FW (2012) Harnessing genomics for delineating conservation units. Trends in Ecology and Evolution 27: 489–496. https://doi.org/10.1016/j.tree.2012.05.012

Frankowski J, Lübke K, Coke M, Weyl OLF (2020) Genetic variability and demographic history of *Anguilla mossambica* (Peters, 1852) from continental Africa and Madagascar. Journal of Fish Biology 96(5): 1251–1259. https://doi.org/10.1111/jfb.14220

Gao TX, Bi XX, Zhao LL, Li CJ (2013) Population genetic structure of roughskin sculpin *Trachidermus fasciatus* based on the mitochondrial Cytb sequence. Acta Hydrobiologica Sinica 37:199–207. [in Chinese with English abstract]

Grant WAS, Bowen BW (1998) Shallow population histories in deep evolutionary linages of marine fishes: Insights from sardines and anchovies and lessons for conservation. Journal of Heredity 89: 415–426. https://doi.org/10.1093/jhered/89.5.415

Guo EM, Liu Y, Liu J, Cui ZX (2010) DNA barcoding discriminates *Pampus minor* (Liu et al. 1998) from *Pampus* species. Chinese Journal of Oceanology and Limnology 28: 1266–1274. https://doi.org/10.1007/s00343-010-9917-1

Inoue JG, Miya M, Tsukamoto K, Nishida M (2001) A mitogenomic perspective on the basal teleostean phylogeny: resolving higher-level relationships with longer DNA sequences. Molecular Phylogenetics and Evolution 20: 275–285. https://doi.org/10.1006/mpev.2001.0970

Jacobsen MW, Da Fonseca RR, Bernatchez L, Hansen MM (2016) Comparative analysis of complete mitochondrial genomes suggests that relaxed purifying selection is driving high nonsynonymous evolutionary rate of the NADH2 gene in whitefish (*Coregonus* ssp.). Molecular Phylogenetics and Evolution 95: 161–170. https://doi.org/10.1016/j.ympev.2015.11.008

Jin XS, Zhao XY, Meng TX, Cui Y (2005) Biology Resource and Environment in the Bohai Sea and Yellow Sea. Scientific Press, Beijing. [in Chinese]

Jing CS, Zhu XM, Bao XW, Song DH (2011) Three dimensional tidal current numerical simulation based on FVCOM in and around Xiamen Bay. Journal of Applied of Oceanography 30: 103–113. [in Chinese with English abstract]

Lambeck K, Esat T, Potter E (2002) Links between climate and sea levels for the past three million years. Nature 419: 199–206. https://doi.org/10.1038/nature01089

Li Y, Zhou YD, Li PF, Gao TX, Lin LS (2019a) Species identification and cryptic diversity in *Pampus* species as inferred from morphological and molecular characteristics. Marine Biodiversity 49: 2521–2534. https://doi.org/10.1007/s12526-019-00976-6

Li Y, Zhang LY, Loh KH, Feng J, Zheng XQ, Song PQ, Lin LS (2019b) Genetic diversity comparison of *Pampus minor* between Chinese and Malaysian populations inferred from mtDNA Cytb. Pakistan Journal of Zoology 51: 149–157. https://doi.org/10.17582/journal.pjz/2019.51.1.149.157

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Liu J, Li CS (1998) A new pomfret species, *Pampus minor* sp. nov (Stromateidae) from Chinese waters. Chinese Journal of Oceanology and Limnology 16: 280–285. https://doi.org/10.1007/BF02848735

Liu JX, Gao TX, Wu SF, Zhang YP (2007) Pleistocene isolation in the Northwestern Pacific marginal seas and limited dispersal in a marine fish, *Chelon haematocheilus* (Temminck & Schlegel, 1845). Molecular Ecology 16: 275–288. https://doi.org/10.1111/j.1365-294X.2006.03140.x

Mishmar D, Ruiz-Pesini E, Golik P, Macaulay V, Clark AG, Hosseini S, Brandon M, Easley K, Chen E, Brown MD, Sukernik RI, Olckers A, Wallace DC (2003) Natural selection shaped regional mtDNA variation in humans. Proceedings of the National Academy of Sciences 100: 171–176. https://doi.org/10.1073/pnas.0136972100

Ohdachi S, Masuda R, Abe H, Adachi J, Dokuchaev NE, Haukisalmi V, Yoshida MC (1997) Phylogeny of Eurasian soricine shrews (Insectivora, Mammalia) inferred from the mitochondrial Cytochrome *b* gene sequences. Zoological Science 14: 527–532. https://doi.org/10.2108/zsj.14.527

Petit JR, Jouzel J, Raynaud D, Barkov NI, Barnola JM, Basile I, Bender M, Chappellaz J, Davis M, Delaygue G, Delmotte M, Kotlyakov VM, Legrand M, Lipenkov VY, Lorius C, PÉpin L, Ritz C, Saltzman E, Stievenard M (1999) Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. Nature 399: 429–436. https://doi.org/10.1038/20859

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28: 2731–2739. https://doi.org/10.1093/molbev/msr121

Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution 9: 552–569. https://doi.org/10.1093/oxfordjournals.molbev.a040727

Rosel PE, Haygood MG, Perrin WF (1995) Phylogenetic relationships among the true porpoises (Cetacea: Phocoenidae). Molecular Phylogenetics and Evolution 4: 463–474. https://doi.org/10.1006/mpev.1995.1043

Saraste M (1999) Oxidative phosphorylation at the fin de siecle. Science 283: 1488–1493. https://doi.org/10.1126/science.283.5407.1488

Shen KN, Jamandre BW, Hsu CC, Tzeng WN, Durand JD (2011) Plio-Pleistocene sea level and temperature fluctuations in the northwestern Pacific promoted speciation in the globally-distributed flathead mullet *Mugil cephalus*. BMC Evolutionary Biology 11: 1–83. https://doi.org/10.1186/1471-2148-11-83

Stepien CA (1999) Phylogeographical structure of the Dover sole *Microstomus pacificus*: the larval retention hypothesis and genetic divergence along the deep continental slope of the Northeastern Pacific Ocean. Molecular Ecology 8: 923–939. https://doi.org/10.1046/j.1365-294x.1999.00643.x

Sun P, Tang BJ (2018) Low mtDNA variation and shallow population structure of the Chinese pomfret *Pampus chinensis* along the China coast. Journal of Fish Biology 92: 214–228. https://doi.org/10.1111/jfb.13515

Wang P (1999) Response of Western Pacific marginal seas to glacial cycles: paleoceanographic and sedimentological features. Marine Geology 156: 5–39. https://doi.org/10.1016/S0025-3227(98)00172-8

Wright S (1965) The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19: 395–420. https://doi.org/10.1111/j.1558-5646.1965.tb01731.x

Wu RX, Liang XH, Zhuang ZM, Liu SF (2012) Mitochondrial COI sequence variation of silver pomfret (*Pampus argenteus*) from Chinese coastal waters. Acta Zootaxonomic Sinica 37: 480–488. [in Chinese with English abstract]

Xu SY, Sun DR, Song N, Han ZQ, Gao TX, Shui BN (2017) Local adaptation shapes pattern of mitochondrial population structure in *Sebastiscus marmoratus*. Environmental Biology of Fishes 100: 763–774. https://doi.org/10.1007/s10641-017-0602-5

Zhang BD, Xue DX, Wang J, Li YL, Liu BJ, Liu JX (2016) Development and preliminary evaluation of a genomewide single nucleotide polymorphisms resource generated by RAD-seq for the small yellow croaker (*Larimichthys polyactis*). Molecular Ecology Resources 16: 755–768. https://doi.org/10.1111/1755-0998.12476

Zhao F, Dong YH, Zhuang P, Zhang T, Zhang, LZ, Shi ZH (2011b) Genetic diversity of silver pomfret (*Pampus argenteus*) in the Southern Yellow and East China Seas. Biochemical Systematics and Ecology 39: 145–150. https://doi.org/10.1016/j.bse.2011.02.002

Zhao F, Zhuang P, Zhang L, Shi ZH (2011a) Population genetic structure of *Pampus argenteus* in the Southern Yellow and East China Sea based on the mitochondrial Cytb sequence. Acta Hydrobiologica Sinica 35: 745–752. [in Chinese with English abstract]